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Identification and characterization of chicken interleukin-16 cDNA

Wongi Min, Hyun Soon Lillehoj*

Parasite Biology, Epidemiology and Systematics Laboratory, Animal and Natural Resources Institute, Beltsville Agricultural Research service, US Department of Agriculture, Agricultural Research Service, Bldg 1040, BARC-East, Beltsville, MD 20705, USA

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Abstract

Interleukin-16 is an inflammatory cytokine synthesized as a precursor protein (pro-IL-16). Based on sequence data from an EST cDNA library prepared from intestinal intraepithelial lymphocytes of *Eimeria*-infected chickens, we identified a cDNA that contained a full-length open reading frame of pro-IL-16. The encoded protein, predicted to consist of 607 amino acids, showed 86% sequence identity to duck pro-IL-16 and 49–52% identity to various mammalian homologues. By Northern blot analysis, IL-16 transcripts were identified in chicken lymphoid tissues but none of the non-lymphoid tissues examined. A recombinant protein containing the 149 C-terminal amino acids of pro-IL-16, expressed in COS-7 cells, showed chemoattractant activity for splenic lymphocytes.

Keywords: Chicken; Interleukin-16; Chemotaxis

1. Introduction

Interleukin-16 (IL-16) was originally described as a lymphocyte chemoattractant factor synthesized by $CD8^+$ and $CD4^+$ T cells and released in response to antigens, mitogens, histamine or serotonin [1]. Further analysis indicated that IL-16 is generated by B cells, mast cells, epithelial cells, macrophages, fibroblasts, and eosinophils [1–4]. IL-16 initially is produced as a 67 kDa pro-IL-16 [5] that subsequently is cleaved by caspase-3 producing a 17 kDa secreted form of the cytokine that aggregates to form biologically active homotetramers [6,7]. IL-16 is chemoattractive for $CD4^+$ T cells, eosinophils, and monocytes through a mechanism involving binding to CD4 [6], although recent data suggest that CD4 is not the only receptor for IL-16 function [8]. In addition to its chemotactic function, IL-16 induces the expression of IL-2 receptor alpha and major histocompatibility complex (MHC) class II molecules [6,9]. In contrast, IL-16 inhibits T cell receptor (TCR)/CD3-dependent lymphocyte activation and human immunodeficiency virus (HIV) promoter activity [10,11]. IL-16 expression has been implicated in the inflammatory responses seen in atopic dermatitis [12], asthma [13], multiple sclerosis [14], rheumatoid arthritis [15], systemic lupus erythematosus [16], and bowel disease [17].

The single copy gene encoding human and mouse IL-16 consisted of seven exons and six introns [18] and homologous sequences have been cloned from nonhuman primates, cat, and cow [19–21]. Keane

* Corresponding author. Tel.: +1-301-504-8771; fax: +1-301-504-5103.
E-mail address: hlilleho@anri.barc.usda.gov (H.S. Lillehoj).

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et al. [22] showed cross-species structural and functional conservation between human and murine IL-16. In contrast, due to low sequence homology between avian and mammalian cytokines, many of the former, including IL-16, have yet to be isolated and characterized [23]. In this report, we describe the cloning of a cDNA encoding chicken pro-IL-16 and characterization of the biological activity of its expressed recombinant protein.

2. Materials and methods

2.1. Chickens

Fertilized eggs of White Leghorn SC chickens were obtained from HyVac (Adel, IA) and hatched at the Animal and Natural Resources Institute (Beltsville, MD). Chickens were kept in wire cages and were provided with water and food ad libitum.

2.2. Construction of a cDNA library and cloning of IL-16

Chickens were orally infected at 3 weeks of age with 1×10^4 sporulated oocysts of *E. acervulina* or *E. maxima*and again at 9 weeks of age with 2×10^4 sporulated oocysts. Intestinal intraepithelial lymphocytes (IELs) were isolated 4 days following the primary and secondary infections according to methods decribed previously [24]. Total RNA was extracted using TRIzol (Life Technologies, Gaithersburg, MD), poly(A)$^+$ RNA prepared using the PolyA Tract mRNA Isolation System IV (Promega, Madison, WI) and reverse-transcribed with an oligo(dT) primer and Superscript II (Life Technologies) reverse transcriptase. The resulting cDNAs were directionally cloned into the *Sal* I and *Not* I sites of the vector, pCMV-SPORT 6 (Life Technologies). From this library, more than 30,000 expressed sequenced tag (EST) clones have been sequenced using high-throughput sequencing technology (unpublished data). Two EST clones (G24, J10) were identified based on sequence homology to mammalian IL-16 sequences available in GenBank using the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/). The G24 and J10 clones, which were not full-length, showed significant homology to the 5′ and 3′ regions of mammalian IL-16, respectively. These two EST clones were completely sequenced and primers (5′-AGCAGCTCAGAGGCAGTTCTG and 5′-TATACA GTTTCAGAGTACCTC) synthesized from their sequences were used to dentify an EST clone (http://www.chickest.udel.edu) designated as pgn1c.pk009.n18 which represents a full-length pro-IL-16 cDNA by PCR. PCR was performed as follows: 1 cycle for 4 min at 94 °C, 30 cycles for 30 s at 55 °C, 1 min at 72 °C and 30 s at 94 °C and a final extension for 5 min at 72 °C.

2.3. Northern blot analysis

Poly(A)$^+$ RNA was isolated using the PolyA Tract mRNA Isolation System IV (Promega, Madison, WI) from total RNA of kidney, bursa, heart, spleen, cecatonsil, thymus, thigh muscle of 10-week-old chickens, subjected to electrophoresis through 1% agarose (NorthernMax-Gly Kit, Ambion, Austin, TX), and transferred to a positively charged nylon membrane (Roche Diagnostics Corporation, Indianapolis, IN). The blot was hybridized with a digoxigenin-labeled chicken IL-16 cDNA probe in DIG Easy Hyb solution (Roche Diagnostics Corporation) at 55 °C overnight. Labeled probe was generated by PCR with the same primers as described above. The blot was washed twice in 2 × SSC and 0.1% SDS for 15 min at room temperature followed twice by 0.1 × SSC and 0.1% SDS for 15 min at 55 °C. Detection was carried out with the DIG High Prime DNA Labeling and Detection Starter Kit (Roche Diagnostics Corporation) according to the manufacturer's instruction. As a control, the chicken β-actin gene was amplified by PCR with the primers 5′-TCTGGTGGTACCACAAT GTACCCT and 5′-CCAGTAATTGGTACCGGCT CCTC.

2.4. Expression of IL-16 recombinant protein in COS-7 cells and Western blot analysis

The nucleotide sequence corresponding to the 149 C-terminal residues (459–607) of chicken pro-IL-16 was amplified by PCR using the following primers: forward, 5′-GATCGGATCCCGATGCCTCAGT GATCATGCG; reverse, 5′-GATCGCGGCCGCTTA $(ATG)_6$CAAAGTTTCAGATGCCTTCTT (6 copy histidine tag indicated by subscript). The PCR

products were digested with *Bam* HI and *Not* I and cloned into the corresponding restriction endonuclease sites of pcDNA3 (Invitrogen). COS-7 cells in 100 mm dishes were transiently transfected with 10 μg of plasmid DNA (IL-16-His) or empty pcDNA3 vector (negative control) using Lipofectamine (Invitrogen), incubated for 48 h at 37 °C in Iscove's Modified Dulbecco's Medium (IMDM, Invitrogen) supplemented with 2 mM L-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, 5 μg/ml 5-fluorocytosine, 2×10^{-6} M 2-mercaptoethanol, and 1 mM sodium pyruvate (all from Sigma). The supernatants of transfected COS-7 cells were harvested and concentrated 10-fold by ultrafiltration using a 10,000 molecular weight cutoff membrane (YM-10; Amicon Centriplus; Millipore, Bedford, MA). The concentrated supernatants and cell lysates were heated at 94 °C for 4 min in SDS–PAGE sample buffer (0.125 M Tris-HCl, pH 6.8, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol, 0.004% bromophenol blue), resolved on 10% SDS–PAGE gels, proteins electrophoretically transferred to nitrocellulose (Immobilon-P, Millipore), blocked with 1% nonfat dry milk in Dulbecco's PBS at 4 °C overnight, and incubated with monoclonal anti-polyhistidine antibody conjugated with peroxidase (Sigma) for 40 min at room temperature. The membrane was washed five times with PBS, five times with distilled water, and developed with SigmaFast DAB peroxidase substrate (Sigma).

2.5. Chemotaxis assay

Chemotactic activity of the IL-16 recombinant protein was assayed by quantification of cell migration using a Tanswell chamber (5.0 μm pore size; Costar). Splenic lymphocytes (5×10^6 cells/ml) were added to the upper chamber well of the Transwell. Concentrated chicken recombinant IL-16 which was expressed in COS-7 cells was added to the lower chamber well and incubated for 2 h at 37 °C in a 5% CO_2 incubator. The Transwell was removed and the number of cells in the lower well was determined by light microscopy. The same volume of the concentrated supernatant of COS-7 cells transfected with the empty pcDNA3 vector was used as control. Student's *t*-test was used to compare differences between means, which were considered significant at $p < 0.05$. Splenic lymphocytes were prepared from chickens of 10–15 weeks of age by gently pushing through a Cell Strainer (Becton Dickinson Labware, Franklin Lakes, NJ) to obtain a single-cell suspension in Hanks' balanced salt solution (HBSS, Sima). Cell suspension was overlaid onto Histopaque-1077 (Sigma) density gradient medium and centrifuged at 200*g* for 25 min at the room temperature. Lymphocytes at the interface were collected, washed three times in HBSS and suspended in IMDM supplemented with 2 mM L-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, 5 μg/ml 5-fluorocytosine, $2 \times 10^{\times 6}$ M 2-mercaptoethanol, and 1 mM

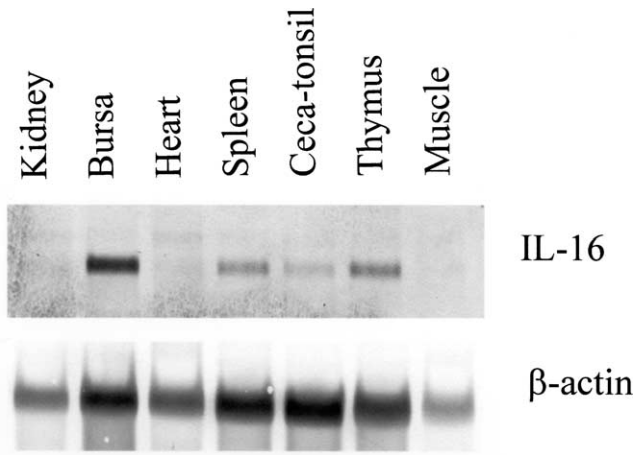

Fig. 1. Northern blot analysis of chicken IL-16 mRNA. Poly $(A)^+$ RNA was isolated from kidney, bursa, heart, spleen, ceca-tonsil, thymus, thigh muscle of 10-week-old chickens and hybridized to digoxigenin-labeled chicken IL-16 cDNA or β-actin probes.

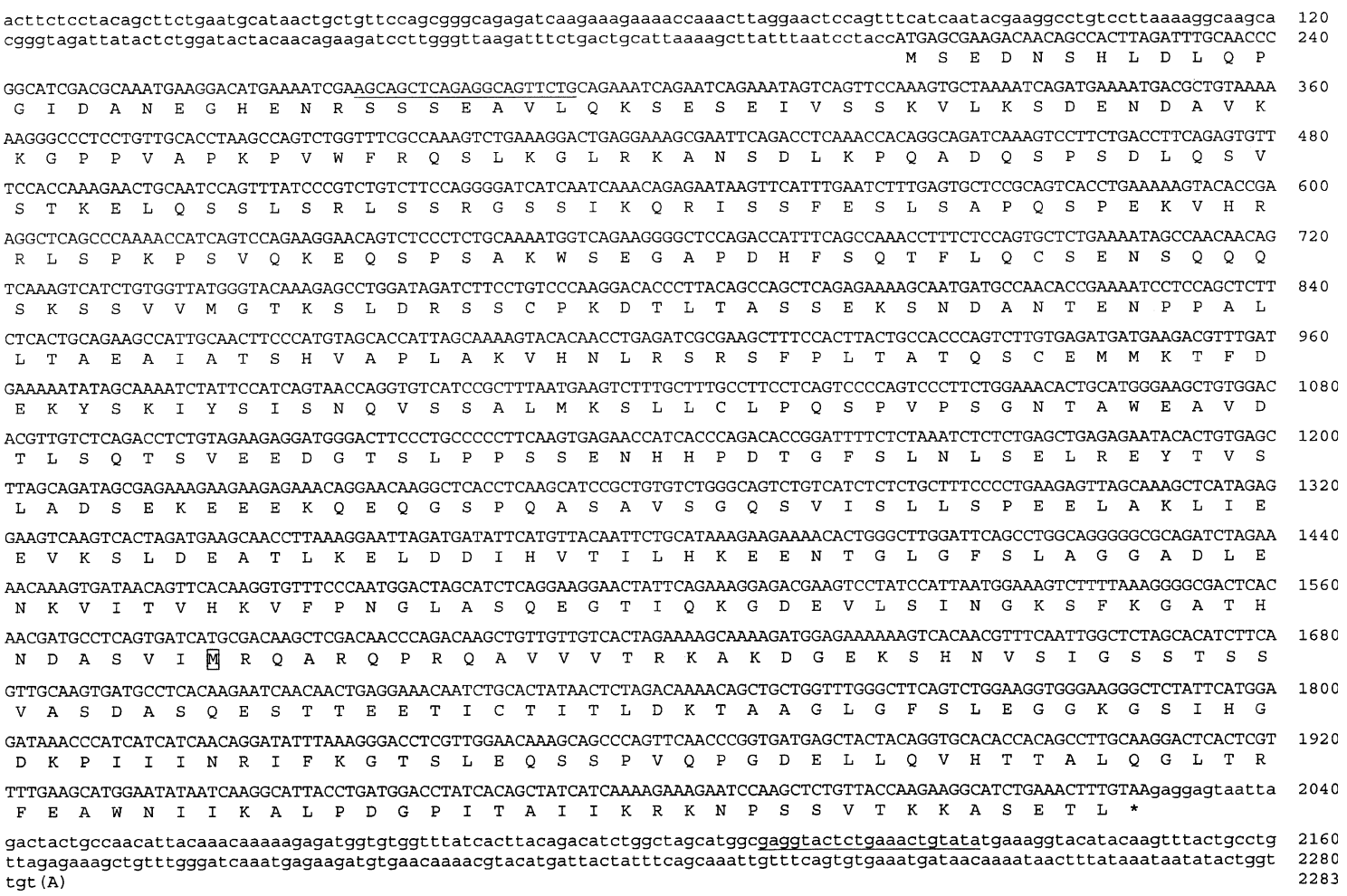

Fig. 2. Nucleotide and deduced amino acid sequences of chicken pro-IL-16. The methionine at position 459 (marked with a box) was used as the translation initiation site for expressing recombinant IL-16. The primers used to identify the full-length EST clones and the probe for Northern blot are underlined. The (A) represents the poly A tail.

sodium pyruvate. Viable cells were counted by trypan blue dye exclusion assay. COS-7 cells were transfected with either IL-16-His plasmid or empty pcDNA3 vector and incubated for 48 h at 37 °C in supplemented IMDM as described above. Supernatants of COS-7 cells were collected, concentrated, and used for assay.

3. Results

3.1. IL-16 mRNA expression

From our EST cDNA library, we identified two partial clones, G24 and J10, encoding polypeptides with significant homology to the 5′ and 3′ regions of mammalian IL-16, respectively. A 1.8 kb fragment was generated by PCR with cDNA synthesized from normal splenic lymphocytes as template and using primers as described in Section 2. The fragment was labeled for Northern blot analysis and hybridized with mRNA from a variety of chicken tissues (Fig. 1). A transcript of approximately 2.5 kb was expressed abundantly in the bursa of Fabricius and moderately in the spleen, ceca/tonsil, and thymus. However, little or no hybridization was observed in the kidney, heart, or muscle. Thus, chicken IL-16 mRNA expression was mainly restricted to lymphoid tissues.

3.2. Chicken IL-16 cDNA

Using a BLAST search of a chicken EST database (http://www.chickest.udel.edu) and PCR, an EST clone designated as pgn1c.pk009.n18 which contains full-length pro-IL-16 cDNA was identified and completely sequenced (Fig. 2). The IL-16 cDNA (GenBank accession no. AJ508678) encodes a polypeptide of 607 amino acids with a predicted molecular mass of 65.3 kDa and an expected isoelectric point of 5.7. As shown in Fig. 3(A), comparison of amino acid sequences indicated that chicken pro-IL-16 shared 86% identity to duck pro-IL-16 (GenBank accession no. AF294321), 52% to human pro-IL-16, and 49% to mouse pro-IL-16. Chicken pro-IL-16 also shared 50-52% identity to monkeys and chimpanzee pro-IL-16 [19]. Evolutionary distance analysis was carried out on amino acid sequences encoded by avian and various mammalian pro-IL-16. The phylogenetic tree showed the expected clustering, confirming the highest similarity between chicken and duck IL-16 (Fig. 3(B)). Chicken pro-IL-16 contains PDZ-like domains harboring a conserved GLGF (Gly-Leu-Gly-Phe) amino acid sequence [25,26]. When PDZ-like domains of chicken pro-IL-16 were compared with those of mouse and human pro-IL-16, the PDZ-1, PDZ-2 and PDZ-3 domains showed 59–63%, 85–87% and 75–77% identity, respectively.

3.3. Chemoattractant activity of IL-16

To assay the biological function of the product of the cloned IL-16 cDNA, we generated an IL-16-His construct haboring the 149 C-terminal residues (amino acids 459–607) of chicken pro-IL-16. The corresponding C-terminal region of human IL-16 includes the PDZ -3 domain and possesses chemotactic activity [27]. The culture supernatants of COS-7 cells transfected with the IL-16-His construct was assayed for chemotactic activity using chicken splenic lymphocytes. As shown in Fig. 4(A), supernatants from COS-7 cells transfected with the IL-16-His construct, but not the empty pcDNA3 plasmid vector, were chemotactic. To further characterize the protein generated by the IL-16-His construct, Western blot analysis was carried out using an anti-polyhistidine antibody. As shown in Fig. 4(B), a 18 kDa band was detected both in the culture supernatant and lysate of COS-7 cells transfected with IL-16-His construct but not the pcDNA3 vector.

4. Discussion

With the recent availability of a large number of chicken EST clones [28–30], it is now possible to isolate and identify novel genes relevant to the avian immune system. In this study, we used this approach to isolate and initially characterize a cDNA encoding IL-16 using an EST cDNA library prepared from IELs of an *Eimeria*-infected chicken. Our major findings delineated here are that the chicken IL-16 transcript was mainly restricted to lymphoid tissues and recombinant IL-16 expressed in COS-7 cells displayed chemotactic activity.

The bursa of Fabricius is a gut-associated lymphoid tissue and the central organ for B cell

A

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                                        ***   *  ***     *              *          * **     *********** **********
chicken     ---------------------------MSEDNSHLDLQPGIDANEGHENRSSSEAVLQKSESEIVSSKVLKSDENDAVKKGPPVAPKPVWFRQSLKGLRKANSDLKPQADQSPSDLQSVS 93
duck        ---------------------------MSEDNSHLDLQPGINTNEENQNRSSSEAVLQKSESEIVSSKVLKSDENDIVKKGPPVAPKPAWFRQSLKGLRKANSDLKTQADQSPPGLQSIS 93
mouse       MDYSFDITAEDPWVRISDCIKNLFSPIMSENHSHTPLQPNTSLGEEDGTQGCPEGGLSKMDAANGAPRVYKSADGSTVKKGPPVAPKPAWFRQSLKGLRNRAPDPRRPPEVASAIQPTPV 120
human       MDYSFDTTAEDPWVRISDCIKNLFSPIMSENHGHMPLQPNASLNEEEGTQGHPDGTPPKLDTANGTPKVYKSADSSTVKKGPPVAPKPAWFRQSLKGLRNRASEPRGLPDPALSTQPAPA 120

                      **    * *******     * *       *  *           *   *                    *              *  *
chicken     TKELQSSLSRLSSRGSSIKQRISSFESLSAPQSPEKVHRRLSPKPSVQKEQSPSAKWSEGAPDHFSQTFLQCSENSQQQSKSSVVMG--TKSLDRSSCPKDTLTASSEKSNDANTENPPA 211
duck        TKELQSSLSCLSSRGSSIKQRISSFESLSAPQSPEKVHRSFSLKTSVQKEHPPSPKGSEGSPTHFNQAFLKCSENSQQQSKSSVVTG--AKSLDRTSPPKEALAASSEESNNANPENSSG 211
mouse       SRDPPGPQPQASSS---IRQRISSFENFGSSQLPDRGVQRLSLQPSSGETTKFPGKQDGGRFSGLLGQGATVTAKHRQTEVESMSTTFPNSSEVRDPGLPESPPPSQRPSTKALS--PDP 235
human       SREHLGSHIRASSSSSSIRQRISSFETFGSSQLPDKGAQRLSLQPSSGEAAKPLGKHEEGRFSGLLGRGAAPTLVPQQPE-QVLSSGSPAASEARDPGVSESPPPGRQPNQKTFPPGPDP 239

            **                *    * ******  ****  *  *** ** **** * *** ******** *              *  *           *    **************
chicken     LLTAEAIATS-HVAPLAKVHNLRSRSFPLTATQSCEMMKTFDEKYSKIYSISNQVSSALMKSLLCLPQSPVPSGNTAWEAVDTLSQTSVEEDGTSLPPSSENHHPDTGFSLNLSELREYT 330
duck        LLTTETVPTS-RALSVTKAHNLRSRSFPLTSTQSCEMMKTYDEKYSKIYSISSQVSSALMKSLLCLPQSPVPSGNSAWEALETLSQSSVEEDGTSLLPASENHHMDTGFSLNLSELREYT 330
mouse       LLRLLTTQSEDTQGPGLKMPSQRARSFPLTRTQSCET-KLLDEKASKLYSISSQLSSAVMKSLLCLP-SSVSCGQITCIPKERVSPKSPCNNSSAAEGFGEAMASDTGFSLNLSELREYS 353
human       LLRLLSTQAEESQGPVLKMPSQRARSFPLTRSQSCET-KLLDEKTSKLYSISSQVSSAVMKSLLCLP-SSISCAQTPCIPKAGASPTSSSNEDSAANGSAETSALDTGFSLNLSELREYT 357

              *     *          *     ********* *** ******  *******  * **********  ********* ********** ********** ********* **
chicken     VSLADSEKEEEKQEQGSPQASAVSGQSVISLLSPEELAKLIEEVKSLDEATLKELDDIHVTILHKEENTGLGFSLAGGADLENKVITVHKVFPNGLASQEGTIQKGDEVLSINGKSFKGA 450
duck        VSLADSEKEEEKQEHGSPQASGVSGQSVISLLSPEELAKLIEEVKSLDEATLKEFDDIHVTILHKEEDTGLGFSLAGGVDLENKVITVHKVFPNGLASQEGTIQKGDEVLSINGKSLKGA 450
mouse       EGLTEP-GETEDRNHCPSQA----GQSVISLLSAEELEKLIEEVRVLDEATLKQLDSIHVTILHKEEGAGLGFSLAGGADLENKVITVHRVFPNGLASQEGTIQKGNEVLSINGKSLKGA 468
human       EGLTEA-KEDDDGDHSSLQS----GQSVISLLSSEELKKLIEEVKVLDEATLKQLDGIHVTILHKEEGAGLGFSLAGGADLENKVITVHRVFPNGLASQEGTIQKGNEVLSINGKSLKGT 472
                        PDZ-1                                                                                                PDZ-2

            ** **  * **** ***** ***                        * *** *  *  * * ** ** *  ************* *****  *******   **   ****** **   ** *
chicken     THNDASVIMRQARQPRQAVVVTRK--AKDGEKSHNVSIGSSTSSVASDASQESTTEETICTITLDKTAAGLGFSLEGGKGSIHGDKPIIINRIFKGTSLEQSSPVQPGDELLQVHTTALQ 568
duck        THNDASAIMRQARQPRQAVVVTRK--PKDGEK-NSISIDSSTSSVASDASQDSATEDTICTVTLEKTPAGLGFSLEGGKGSIHGDKPIVINRIFKGTALEQSSTVQPGDELLQVHTTAMQ 567
mouse       THNDALAILRQARDPRQAVIVTRRTTVEATHDLNSSTDSAASASAASDISVES-KEATACTVTLEKTSAGLGFSLEGGKGSLHGDKPLTINRIFKG--TEQGEMVQPGDEILQLAGTAVQ 585
human       THHDALAILRQAREPRQAVIVTRKLTPEAMPDLNSSTDSAASASAASDVSVESTAEATVCTVTLEKMSAGLGFSLEGGKGSLHGDKPLTINRIFKGAASEQSETVQPGDEILQLGGTAMQ 592
                                                                                                                    PDZ-3

            ********* ******** *  * *          *
chicken     GLTRFEAWNIIKALPDGPITAIIKRKNPSSVTKKASETL- 607
duck        GLTRFEAWNIIKALPDGPITAIIKRRNPSSVTTKASETL- 606
mouse       GLTRFEAWNVIKALPDGPVTIVIRRTSLQCKQTTASADS- 624
human       GLTRFEAWNIIKALPDGPVTIVIRRKSLQSKETTAAGDS- 631
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Fig. 3. Sequence comparison and phylogenetic tree of proteins encoded by pro-IL-16. (A) Sequences were aligned using the CLUSTAL W (1.82) program (www.ebi.ac.uk/clustalw/). Asterisks (*) indicate identical residues among sequences. The three PDZ domains are underlined. (B) Phylogenetic tree was constructed by neighbor joining method using amino acid sequences aligned with CLUSTAL W. Duck IL-16 (accession no. AF294321); mouse IL-16 (accession no. NM_010551); human IL-16 (accession no. NM_004513); aotus IL-16 (accession no. AF017110); saimiri IL-16 (accession no. AF017109); chimpanzee IL-16 (accession no. AF007879); cynomolgus IL-16 (accession no. AF017108); rhesus IL-16 (accession no. AF017107) and African green monkey (AGM) IL-16 (accession no. AF017106).

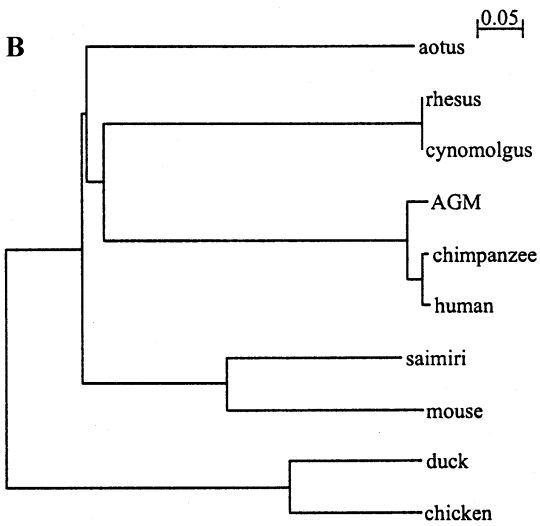

Fig. 3 (*continued*)

lymphopoiesis, lymphocyte maturation, and generation of antibody diversity [31]. B lymphocytes comprise 90% of the total bursal lymphocyte pool [32] and interactions between B lymphocytes and dendritic cells is an important factor in the proliferation, differentiation, and isotype switching [33,34]. In mammals, B lymphocytes express IL-16 transcripts and bioactive IL-16 protein, a major chemotactic factor. Supernatants from unstimulated B lymphocytes attract dendritic cells [4]. In our study, Northern blot analysis (Fig. 1) showed that IL-16 transcript is highly expressed in the bursa of Fabricius compared to other lymphoid tissues. This finding suggests a possibility of IL-16 involvement in the development of B-cells. However, the relevance of our finding to chicken B lymphocyte development in the bursa remains to be investigated.

By sequence analysis, the predicted amino acid sequence of chicken IL-16 contains no consensus secretory leader sequence as is also the case for human and mouse IL-16 [19,35]. In the 3′ untranslated region, no AU-rich (ATTTA) mRNA instability sequences were noted in chicken IL-16 which is similar to the mouse gene but different from human IL-16 [19,35,36]. In addition, chicken pro-IL-16, similarly to the predicted duck protein, was 17–25 amino acids shorter than mammalian pro-IL-16. To localize where this potential difference was occurred, we carried out sequence alignment of the proteins encoded by avian and mammalian pro-IL-16 genes and identified the 27 amino acid short in the 5′ region of the avian genes. Since alternative translation initiations starting at non-ATG sites have been reported [37,38], the sequence upstream of the proposed ATG of chicken IL-16 was translated and compared to that of mouse IL-16. The short region of chicken IL-16 showed 66% identity (18/27) with the equivalent region of mouse IL-16. In light of the high score identity, there is a possibility that chicken pro-IL-16 protein may be initiated at GTA, a non-ATG start codon at position 124 bp. While chicken pro-IL-16 showed 49–52% identity with mammalian pro-IL-16 homologs, the PDZ-like domains of chicken pro-IL-16 showed a higher degree of conservation, particularly in the second and third PDZ-like domains. Interestingly, a neuronal IL-16 precursor cloned from adult mouse cerebellum contains four PDZ domains [39], although pro-IL-16 derived from peripherial blood mononuclear cells or splenocytes contains three such domains [19,22]. PDZ domains were first identified as ~90 amino acid-long repeated sequence in the synaptic protein PSD-95. These domains have also been termed Discs large homology repeat or GLGF repeats. PDZ domains are protein–protein interaction domains that bind in sequence-specific fashion to short C-terminal peptide or internal peptides. They have been frequently found in a wide variety of proteins in

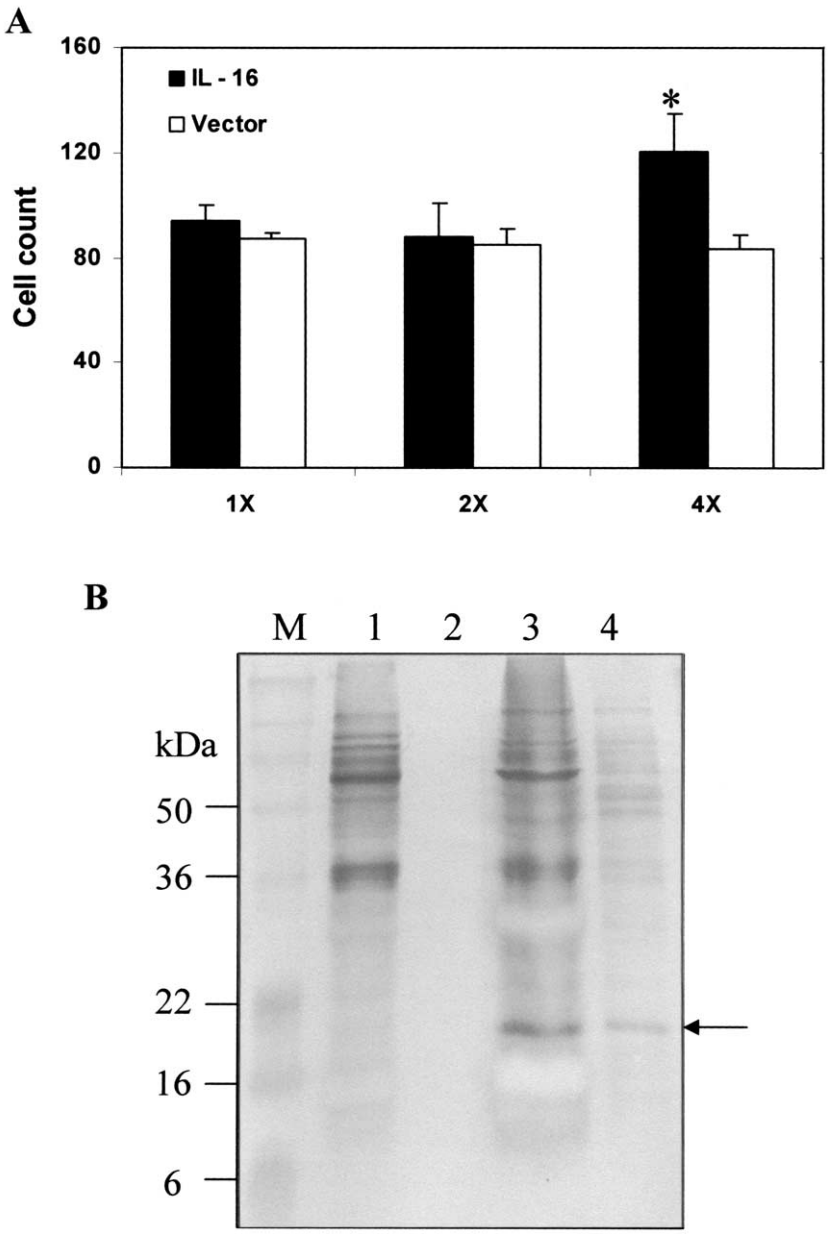

Fig. 4. Characterization of recombinant IL-16 expressed in COS-7 cells. (A) Recombinant IL-16 induced chemotactic migration of splenic lymphocytes. Splenic lymphocytes were added to the upper chamber well of a Transwell containing a 5.0 μm pore membrane. Concentrated chicken recombinant IL-16 which was expressed in COS-7 cells was added to the lower chamber well. The same volume of the concentrated supernatant of COS-7 cells transfected with the empty pcDNA3 vector was used as control. Migrated cell numbers after 2 h incubation were counted by light microscopy. The data showed the mean ± SD of a representative result. The experiment was performed three times with duplicate samples at least and yielded similar results. Comparisons between control and experimental condition were analyzed by Student's *t*-test. The asterisk (*) indicates statistical significance ($p < 0.05$) for a difference in splenic lymphocyte migration. *X* indicates the degree of concentration of supernatants of COS-7 cells. (B) Western blot analysis of recombinant IL-16. The IL-16-His construct (lanes 3 and 4) or pcDNA3 vector alone (lanes 1 and 2) as control was transfected into COS-7 cells. Cell lysates (lanes 1 and 3) and supernatants (lanes 2 and 4) were resolved by SDS–PAGE, transferred to nitrocellulose by electroblotting, probed with a monoclonal anti-polyhistidine antibody conjugated with peroxidase, and visualized by Sigma Fast DAB peroxidase substrate. M indicates molecular weight marker.

diverse organisms as multiple copies or association with other protein-binding motifs. PDZ-containing proteins play an important role in the transport, localization and assembly of large protein complexes [25,26].

Pro-IL-16 can be processed by caspase-3 to give rise to a secreted C-terminal portion which functions both as a chemoattractant and a suppressive factor for human immunodeficiency virus replication [6,7,10, 11,27,40]. This secreted C-terminal portion constitutes the mature form of IL-16 and consists of a central PDZ domain flanked by NH2-terminal and C-terminal tails. Although both tails of the mature IL-16 are involved in IL-16 functional activities, only the C-termial tails are critical for chemoattractant activity [27]. We have now confirmed this result in the chicken cytokine by our demonstration that an IL-16-His construct consisting of the 149 C-terminal residues of chicken pro-IL-16 displayed chemotactic activity for splenic lymphocytes. The residual NH2-terminal domain remaining after removal of the chemotactic peptide contains two PDZ domains and a classical bipartite nuclear localization sequence (NLS) allowing it to translocate into the nucleus and induce G_0/G_1 cell cycle arrest [41]. As shown in Fig. 3(A), the corresponding region between amino acids 52–74 (KKGPPVAPKPVWFRQSLKGLRKA) of chicken pro-IL-16 showed 86% homology to the human and mouse NLS suggesting that it may serve a similar function in avians. Current studies in our laboratory are directed at further defining the structural and functional similarities of chicken and mammalian IL-16 and related cytokines.

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